Clinical and metabolic response to probiotic administration in patients with major depressive disorder: a randomized, double-blind, placebo-controlled trial

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Clinical and metabolic response to probiotic administration in patients with major depressive disorder: a randomized, double-blind, placebo-controlled trial

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Running Title: Probiotic administration and depression

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Abstract

Background: We are aware of no study examining the effects of probiotic supplementation on symptoms of depression, metabolic profiles, serum high sensitivity C-reactive protein (hs-CRP) and biomarkers of oxidative stress among patients with major depressive disorder (MDD).

Objective: The current study was designed to determine the effects of probiotic intake on symptoms of depression and metabolic status among patients with MDD.

Methods: This randomized double-blind placebo-controlled clinical trial was done among 40 patients aged 20-55 years old with a diagnosis of MDD based on DSM-IV criteria. Patients were randomly allocated into two groups to receive either probiotic supplements (n=20) or placebo (n=20) for 8 weeks. Probiotic capsule was consisted of three viable and freeze-dried strains: Lactobacillus acidophilus \((2\times10^9\ \text{CFU/g})\), Lactobacillus casei \((2\times10^9\ \text{CFU/g})\) and Bifidobacterium bifidum \((2\times10^9\ \text{CFU/g})\). Fasting blood samples were taken at the beginning and end-of-trial to quantify the relevant variables. All participants provided three dietary records (2-week days and 1-week end) and three physical activity records during intervention.

Results: Dietary intakes of study participants were not significantly different between the two groups. After 8 weeks of intervention, patients who received probiotic supplements had significantly decreased Beck Depression Index (BDI) total score (-5.7±6.4 vs. -1.5±4.8, \(P=0.001\)) compared with the placebo. In addition, significant decreases in serum insulin levels (-2.3±4.1 vs. +2.6±9.3 \(\mu\text{IU/mL}, P=0.03\)), homeostasis model assessment of insulin resistance (HOMA-IR) (-0.6±1.2 vs. +0.6±2.1, \(P=0.03\)) and serum hs-CRP concentrations (-1138.7±2274.9 vs. +188.4±1455.5 \(\text{ng/mL}, P=0.03\)) were observed following the probiotic supplementation compared with the placebo. Additionally, taking probiotics resulted in a significant rise in plasma total glutathione (GSH) levels (+1.8±83.1 vs. -106.8±190.7 \(\mu\text{mol/L}, P=0.02\)) compared with the placebo. We did not find any significant change in fasting plasma glucose (FPG), homeostatic
model assessment of Beta cell function (HOMA-B), quantitative insulin sensitivity check index (QUICKI), lipid profiles and total antioxidant capacity (TAC) levels.

**Conclusion:** Probiotic administration among patients with MDD for 8 weeks had beneficial effects on BDI, insulin, HOMA-IR, hs-CRP and GSH concentrations, but did not influence FPG, HOMA-B, QUICKI, lipid profiles and TAC levels.

**KEYWORDS:** Probiotic, glucose metabolism, lipid profiles, oxidative stress, depression
Introduction

Major depressive disorder (MDD) is a complex and multi-factorial disorder that involves marked disabilities in global functioning, anorexia, and severe medical comorbidities [1]. It influences around 20% of the population at some point during the lifetime of an individual [2]. Previous studies have shown a link between metabolic profiles, biomarkers of inflammation, oxidative stress and MDD [1, 3-4]. Depression or depressive episodes may have effects on cortisol dysregulation which might in turn result in the development of insulin resistance in patients with depression [5]. In addition, recent studies have reported that decreased antioxidant levels especially glutathione (GSH) is associated with increased anhedonia severity that subsequently might lead to involvement of neuroinflammation and oxidative stress in MDD [6].

Probiotics are proposed to have a range of health benefits. Their beneficial impacts on a wide range of symptoms have been examined, including the relief of irritable-bowel syndrome, inflammatory bowel disease, the amelioration of lactose intolerance and the prevention of bowel cancer [7-8]. Moreover, emerging research has reported that the microflora of the intestines may affect the immune system and functioning beyond the gut [9]. Probiotics might have favorable effects on mood and psychological problems [10]. In a study by Mohammadi et al. [11], consumption of probiotic yogurt or a multispecies probiotic capsule for 6 weeks had beneficial effects on mental health parameters in petrochemical workers. Others have also reported the favorable effects of probiotic administration in healthy subjects [12] and patients with chronic fatigue syndrome (CFS) [13]. In a study by Benton et al. [14], consumption of probiotic-containing yoghurt improved the mood of those whose mood was initially poor. In addition, improved metabolic status, biomarkers of inflammation and oxidative stress were observed following a two months supplementation with probiotics among pregnant women and patients with type 2 diabetes mellitus (T2DM) [15-16]. However, probiotic supplementation containing
**Lactobacillus rhamnosus** strain GG and bifidobacterium had no beneficial effects in people with schizophrenia after 14 weeks [17].

Probiotics may result in improved depressive symptoms, metabolic status, biomarkers of inflammation and oxidative stress through their effect on neuronal circuits and central nervous system mediated by microbiota-gut-brain axis [18] and through affecting gene expression [19]. In addition, experimental studies in the animal model of depression have demonstrated that the oral administration of a probiotic can increase plasma tryptophan concentrations, decrease serotonin metabolite concentrations in the frontal cortex and dopamine metabolite concentrations in the amygdaloid cortex [20]. However, whether probiotics have direct benefits on depressive symptoms and metabolic status in patients with MDD has to date not been assessed. The current study was, therefore, done to assess the favorable effects of probiotic supplementation on symptoms of depression, parameters of glucose homeostasis, lipid concentrations, biomarkers of inflammation and oxidative stress in patients with MDD.

**Materials and Methods**

**Participants**

Forty patients aged between 20 and 55 years old with MDD were recruited in a randomized, double-blind, placebo-controlled trial from July 2014 to September 2014. To determine the sample size, we applied a randomized clinical trial sample size formula considering type one \( (\alpha) \) and type two errors \( (\beta) \) of 0.05 and 0.20 (power=80%), respectively. Based on a previous study [11], we used a standard deviation (SD) of 18.5 and a difference in mean (d) of 18, considering DASS (depression anxiety and stress scale) as the key variable. This calculation indicated a total of 17 patients for each group. However, we recruited 40 patients with MDD in total (20 patients in each group) to compensate for the probable loss to follow up. The patients with a diagnosis of
MDD, based on DSM-IV criteria and with a score of \( \geq 15 \) on the 17-item Hamilton Depression Rating Scale (HDRS), who were referred from Kargarneghad Hospital, Kashan University of Medical Sciences (KUMS), Kashan, Iran were included in the study. Exclusion criteria were age < 20 and >55 years, those with a history of coronary infarction, angina pectoris, pregnancy or lactation, substance abuse, taking dietary supplements and the intake of probiotic supplements during the previous 2 months. All procedures followed were according to the ethical standards of the responsible committee on human experimentation (institutional and national) and with the Helsinki Declaration. In addition, the ethical committee of KUMS approved the study. All patients provided written informed consent. This study was registered in the Iranian website (www.irct.ir) for registration of clinical trials (IRCT code: IRCT2014060717993N1).

**Study design**

In the current study, patients were randomly allocated into two groups to receive either probiotic supplements (17 females and 3 males: n=20) or placebo (17 females and 3 males: n=20) for 8 weeks. Patients in the probiotic group received one probiotic capsule daily containing *Lactobacillus acidophilus* \( (2 \times 10^9 \text{ CFU/g}) \), *Lactobacillus casei* \( (2 \times 10^9 \text{ CFU/g}) \) and *Bifidobacterium bifidum* \( (2 \times 10^9 \text{ CFU/g}) \). It is well known that it would be more appropriate if the strains used in probiotic supplements for human consumption derived from the human intestinal tract, well characterised, able to outlive the rigors of the digestive tract and possibly colonise, biologically active against the target as well as to be stable and amenable to commercial production and distribution [21]. Due to the lack of evidence about the appropriate dosage of probiotics for patients with MMD, we used the above-mentioned doses based on few previous studies in healthy subjects [11, 14]. Subjects in the placebo group received the placebo that contained starch but no bacteria. The appearance of the placebo was indistinguishable in color,
shape, size, and packaging, smell and taste from the probiotic supplement. All capsules were provided by Tak Gen Zist Pharmaceutical Company, Tehran, Iran, that approved by Food and Drug Administration. Random assignment was performed by the use of computer-generated random numbers. Randomization and allocation were concealed from the researcher and participants until the main analyses were completed. The randomized allocation sequence, enrolling patients and allocating participants to interventions were done by a trained nutritionist at psychiatry clinic. At the beginning of the study, patients were requested not to change their routine physical activity or usual dietary intakes throughout the study and not to consume any supplements other than the one provided to them by the investigators as well as not to take any medications that might affect findings during the 8-wk intervention. Compliance to probiotic and placebo capsules was monitored by asking participants to return the medication containers. All participants provided three dietary records (2-week days and 1-week end) and three physical activity records to make sure that they maintained their usual diet and physical activity during intervention. Both dietary and physical activity records were taken at week 2, 4 and 6 of intervention. To obtain nutrient intakes of participants based on these three-day food diaries, we used Nutritionist IV software (First Databank, San Bruno, CA) modified for Iranian foods.

**Anthropometric assessment**

Body weight and height were determined in an overnight fasting state, without shoes and in a minimal clothing state by the use of a digital scale (Seca, Hamburg, Germany) by a trained nutritionist at psychiatry clinic at the beginning of the study and at end-point. BMI was calculated as weight in kg divided by height in meters squared.

**Outcomes**
In this study, the primary outcome was BDI. Depressed mood was judged with BDI at the beginning and the end of study. BDI is a self-compiled questionnaire of 21 items in multiple choice format [22]. On each item, there are four statements and the subjects were instructed to choose the one that best described their situation during the last 2 weeks. The declarations are given the scores of 0, 1, 2 and 3, with ‘0’ for the ‘normal’ or least depressive statement and ‘3’ for the most depressive statement. We calculated the total BDI score by adding together the scores of each item. Secondary outcomes were fasting plasma glucose (FPG), markers of insulin metabolism, lipid concentrations, serum hs-CRP and biomarkers of oxidative stress including total antioxidant capacity (TAC) and GSH levels. Fasting blood samples (10 mL) were obtained at the baseline and study end-point after 12 h of fasting at Kashan reference laboratory in an early morning after an overnight fast. Blood samples were immediately centrifuged (Hettich D-78532, Tuttlingen, Germany) at 3500 rpm for 10 min to separate serum. Then, the samples were stored at -80°C before analysis at the KUMS reference laboratory. To determine FPG, triglycerides, total-, VLDL-, LDL- and HDL-cholesterol concentrations, we applied commercial kits (Pars Azmun, Tehran, Iran). All inter- and intra-assay CVs for FPG and lipid profiles measurements were less than 5%. To determine serum insulin and hs-CRP concentrations, we used ELISA kits (Monobind, California, USA and LDN, Nordhorn, Germany, respectively). Homeostasis model of assessment of insulin resistance (HOMA-IR), β-cell function (HOMA-B) and quantitative insulin sensitivity check index (QUICKI) were determined based on suggested formulas [23]. Plasma TAC was quantified by the use of FRAP method modified by Benzie and Strain [24] and GSH by the method modified by Beutler et al [25].

Statistical analysis
To determine the normal distribution of variables, we used Kolmogrov-Smirnov test. The analyses were conducted based on intention-to-treat approach (ITT). Missing values were treated based on Last-Observation-Carried-Forward (LOCF) method. To detect differences in general characteristics and dietary intakes between the two groups, we used independent samples Student’s t test. To determine the effects of probiotic administration on markers of insulin metabolism, lipid concentrations, serum hs-CRP and biomarkers of oxidative stress, we used one-way repeated measures analysis of variance. Within-group comparisons (end-point vs. baseline) were done based on paired samples t-test. To control for several confounders, we applied analysis of covariance (ANCOVA) in which the confounding effect of these variables were taken into account. P-value <0.05 was considered as statistically significant. All statistical analyses were done using the Statistical Package for Social Science version 17 (SPSS Inc., Chicago, Illinois, USA).

Results

Among patients in the probiotic group, 3 persons met the exclusion criteria: [withdrawn due to personal reasons (n=3)]. The exclusions in the placebo group were also 2 patients [withdrawn due to personal reasons (n=2)]. Finally, 37 persons [probiotic (n=17) and placebo (n=18)] completed the trial (Fig. 1). However, as the analysis was done based on ITT, all 40 patients with MDD were included in the final analysis. Totally, the rate of compliance in the current study was high, such that more than 90% of capsules were taken throughout the study in both groups.

Baseline and end-of-trial means of weight and BMI were not significantly different between probiotic supplements and placebo groups (Table 1).
No significant change was observed between the two groups in terms of dietary intakes of energy, carbohydrates, proteins, fats, saturated fatty acids (SFA), polyunsaturated fatty acids (PUFA), monounsaturated fatty acids (MUFA), cholesterol, dietary fiber, magnesium, manganese and zinc that were obtained based on three-day dietary records throughout the intervention (Table 2).

After 8 weeks of intervention, patients who received probiotic supplements had significantly decreased BDI score (-5.7±6.4 vs. -1.5±4.8, P=0.001) compared with the placebo (Fig.2). In addition, significant decreases in serum insulin levels (-2.3±4.1 vs. +2.6±9.3 µIU/mL, P=0.03), HOMA-IR (-0.6±1.2 vs. +0.6±2.1, P=0.03) and hs-CRP concentrations (-1138.7±2274.9 vs. +188.4±1455.5 ng/mL, P=0.03) were observed following the supplementation with probiotic compared with the placebo. Additionally, taking probiotics resulted in a significant rise in plasma GSH levels (+1.8±83.1 vs. -106.8±190.7 µmol/L, P=0.02) compared with the placebo.

A trend toward a significant decrease in HOMA-B (-7.1±13.7 vs. +9.8±37.4, P=0.06) and an increase in QUICKI score (+0.009±0.01 vs. -0.003±0.02, P=0.07) was observed after probiotics supplementation (Table 3). We did not find any significant change in FPG, HOMA-B, QUICKI, lipid profiles and TAC levels after supplementation.

Baseline levels of FPG were significantly different between the two groups. Therefore, we controlled the analyses for the baseline levels, age and baseline BMI. However, after this adjustment no significant changes in our findings occurred, except for BDI score (P=0.05) and serum insulin levels (P=0.05) (Table 4).
**Discussion**

In the current study, we examined the beneficial effects of probiotic administration on BDI score, markers of insulin metabolism, lipid profiles, hs-CRP and biomarkers of oxidative stress among patients with MDD. The main findings were that probiotic supplementation led to improved BDI score and insulin function as well as decreased oxidative stress in MDD patients. To the best of our knowledge, this study is the first that reports the effect of probiotic administration on symptoms of depression, metabolic status, biomarkers of inflammation and oxidative stress among patients with MDD.

Patients with MDD are predisposed to some complications including morbidity, mortality [26], increased risk of CVD, dyslipidemia and impaired insulin function [27]. Our study demonstrated that taking probiotic supplements among MDD patients for 8 weeks resulted in a significant decrease in BDI score compared with the placebo. However, few studies have assessed the effects of probiotic supplementation on symptoms of depression. In a study by Rao et al.[28], a significant decrease in anxiety symptoms was observed among those taking the probiotics compared with controls; however, they failed to find any significant effect on BDI score. In addition, supplementation with probiotic sachet containing two strains of *Lactobacillus helveticus* and *Bifidobacterium Longum* (3×10^{12} CFU/1.5 g sachet) for 30 days among healthy persons resulted in a significant improvement in mental health [12]. However, no significant improvement was observed following the supplementation of *Lactobacillus rhamnosus* and *Bifidobacterium animalis* for 14 weeks in schizophrenia patients [17]. The accurate mechanism of probiotics in the brain and its effects on depression is not completely understood. The administration of probiotics might result in improved symptoms of depression through increased plasma tryptophan levels, decreased serotonin metabolite concentrations in the frontal cortex and dopamine metabolite concentrations in the amygdaloid cortex [20]. Various factors including
host physiology, immunology, diet, antibiotic use and enteric infection can affect the gut microbiota composition and its activity. Probiotic bacteria through fermenting dietary ingredients might lead to specific changes in the composition and/or activity of the gastrointestinal microbiota, through which they might result in improved peripheral (gastrointestinal) and central (psychological) symptoms [29]. Probiotics may influence both the enteric nervous system (ENS) and the central nervous system (CNS) in addition to their effects on the mucosal immune system by modifying the gastrointestinal tract (GI) microbiome [29]. In addition, few studies have indicated that probiotics might improve carbohydrate malabsorption [30] that are associated with both the early signs of depression [31] and reduced tryptophan levels [32].

We found that probiotic supplementation for 8 weeks in patients with MDD led to significant decreases in serum insulin concentrations and HOMA-IR compared with the placebo, but it did not affect FPG, HOMA-B, QUICKI and lipid profiles. In agreement with our study, Firouzi et al. [33] conducted a review study on this subject and they found that sixteen, out of seventeen studies in animals, and three out of four studies in humans, had reported significant improvements in at least one glucose homeostasis-related parameter [33]. In addition, in a study by Ejtahed et al [34], probiotic yogurt consumption containing lactobacillus acidophilus and Bifidobacterium lactis for 6 weeks did not affect lipid profiles among patients with T2DM. Some investigators did not observe any beneficial effects of probiotic supplementation on markers of insulin metabolism. For instance, supplementation with the probiotic strain of Lactobacillus casei Shirota for 12 weeks did not improve insulin sensitivity and β-cell function in subjects with metabolic syndrome [35]. The mechanism by which probiotic intake might improve markers of insulin metabolism may be attributed to an increase in hepatic natural killer T-cell number and a reduction in inflammatory signaling [36]. Moreover, conjugated linoleic acid is produced by some species of Lactobacilli including acidophilus, plantarum, paracasei and casei, might up-
regulate adiponectin, down-regulate inflammation, block suppression of glucose transporter type 4 [37]. The different findings might be explained by different study designs, different dosages of probiotics used as well as different participants of the study.

Findings from the current study revealed that taking supplemental probiotics resulted in decreased serum hs-CRP levels in patients with MDD. Supporting our study, Zarrati et al. [38] demonstrated that taking probiotic yogurt containing *Lactobacillus acidophilus, Bifidobacterium animalis* and *Lactobacillus casei* for 8 weeks resulted in a significant decrease in hs-CRP levels among overweight and obese individuals. In addition, a significant decrease in hs-CRP levels was observed following the administration of probiotic yogurt among pregnant women for 9 weeks [39] and patients with established rheumatoid arthritis (RA) [40]. However, an 8-week multispecies probiotic supplementation did not influence CRP levels in polycystic ovary syndrome (PCOS) patients [41]. Hs-CRP, as a marker of systemic inflammation, is a important independent predictor of risk of future myocardial infarction, stroke and peripheral arterial disease [42]. The anti-inflammatory effects of probiotics might be explained by the production of short chain fatty acids (SCFA) in the colon [43] and by the decreased expression of interleukin-6 (IL-6) [44].

The present study showed that patients who received probiotic supplements had significantly increased plasma GSH levels compared with the placebo, but we did not find any effect on TAC levels. Our findings were in accordance with those reported by other researchers, showing increased GSH levels in patients with T2DM after probiotic intake for 8 weeks [15, 45]. Furthermore, a significant increase in GSH concentrations was observed after intake of *Lactobacillus plantarum* in rats for 14 days. However, our previous study among pregnant women revealed that consumption of probiotic yogurt containing two strains of *Lactobacillus acidophilus* and *Bifidobacterium animalis* for 9 weeks did not influence plasma GSH levels
compared with the conventional yogurt [16]. The accurate mechanisms by which intake of probiotic supplements might affect biomarkers of oxidative stress are unknown. The beneficial effects of probiotics on GSH might be explained by the enhanced glutamate-cysteine-ligase activity (GCL), increased mRNA expression of GCL subunits and increased synthesis of GSH [46].

Some limitations of the current study need to be considered: We were not able to assay the effect of probiotic supplementation on other biomarkers of inflammation and oxidative stress. Another limitation of the study was the duration of intervention. We were unable to administer probiotic supplements for more than 8 weeks. Long-term interventions would be required to confirm the beneficial effects on lipid profiles. In addition, we don't know if the treatment effect observed in our study was due to the effect of which strain. Therefore, further studies are needed with single strain used in the current study in order to evaluate the beneficial effects on symptoms of depression and metabolic status among patients with MDD. In the current study, one depression variable was used to estimate sample size because the largest sample size was obtained when we used this variable. Therefore, the sample size obtained based on this variable were covering the required sample size for all other variables. The study power was 80%. Despite this, we agree that large-scale trials would be needed to confirm our findings.

Taken together, probiotic administration among patients with MDD for 8 weeks had beneficial effects on BDI, insulin, HOMA-IR, hs-CRP and GSH levels, but did not influence FPG, HOMA-B, QUICKI, lipid profiles and TAC levels.
Acknowledgment

The present study was supported by a grant from the Vice-chancellor for Research, KUMS, and Iran. The authors would like to thank the staff of Kargarneghad Hospital (Kashan, Iran) for their assistance in this project.

Conflicts of interest

None of the authors had any personal or financial conflict of interest.

Funding

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References


Table 1

General characteristics of the study participants

<table>
<thead>
<tr>
<th></th>
<th>Placebo group (n=20)</th>
<th>Probiotic group (n=20)</th>
<th>P (^1)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (y)</td>
<td>36.2±8.2</td>
<td>38.3±12.1</td>
<td>0.52</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>160.9±4.9</td>
<td>163.3±9.5</td>
<td>0.31</td>
</tr>
<tr>
<td>Weight at study baseline (kg)</td>
<td>68.0±11.5</td>
<td>72.6±11.3</td>
<td>0.21</td>
</tr>
<tr>
<td>Weight at end-of-trial (kg)</td>
<td>68.7±10.5</td>
<td>72.5±11.1</td>
<td>0.28</td>
</tr>
<tr>
<td>Weight change (kg)</td>
<td>0.7±2.7</td>
<td>-0.1±1.6</td>
<td>0.26</td>
</tr>
<tr>
<td>BMI at study baseline (kg/m(^2))</td>
<td>26.3±4.1</td>
<td>27.6±6.0</td>
<td>0.42</td>
</tr>
<tr>
<td>BMI at end-of-trial (kg/m(^2))</td>
<td>26.5±3.9</td>
<td>27.5±5.9</td>
<td>0.53</td>
</tr>
<tr>
<td>BMI change (kg/m(^2))</td>
<td>0.2±1.0</td>
<td>-0.1±0.6</td>
<td>0.23</td>
</tr>
</tbody>
</table>

\(^1\) Data are means± SDs.

\(^1\) Obtained from independent t test.
Table 2

Dietary intakes of study participants throughout the study

<table>
<thead>
<tr>
<th></th>
<th>Placebo group (n=20)</th>
<th>Probiotic group (n=20)</th>
<th>P&lt;sup&gt;1&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Energy (kcal/d)</td>
<td>2222±97</td>
<td>2268±112</td>
<td>0.17</td>
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<tr>
<td>Carbohydrates (g/d)</td>
<td>305.9±46.2</td>
<td>318.2±38.4</td>
<td>0.36</td>
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<tr>
<td>Protein (g/d)</td>
<td>85.2±25.9</td>
<td>80.9±9.4</td>
<td>0.48</td>
</tr>
<tr>
<td>Fat (g/d)</td>
<td>75.9±17.5</td>
<td>78.1±14.3</td>
<td>0.66</td>
</tr>
<tr>
<td>SFA (g/d)</td>
<td>22.2±7.1</td>
<td>23.6±6.9</td>
<td>0.52</td>
</tr>
<tr>
<td>PUFA (g/d)</td>
<td>25.6±4.5</td>
<td>26.1±5.9</td>
<td>0.77</td>
</tr>
<tr>
<td>MUFA (g/d)</td>
<td>20.3±7.1</td>
<td>19.8±5.0</td>
<td>0.79</td>
</tr>
<tr>
<td>Cholesterol (mg/d)</td>
<td>240.2±185.5</td>
<td>202.2±119.5</td>
<td>0.44</td>
</tr>
<tr>
<td>TDF (g/d)</td>
<td>16.9±3.5</td>
<td>16.8±4.4</td>
<td>0.95</td>
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<tr>
<td>Magnesium (mg/d)</td>
<td>249.1±38.6</td>
<td>256.3±40.9</td>
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</tr>
<tr>
<td>Manganese (mg/d)</td>
<td>1.9±0.8</td>
<td>2.0±0.7</td>
<td>0.70</td>
</tr>
<tr>
<td>Zinc (mg/d)</td>
<td>8.9±2.9</td>
<td>9.4±2.8</td>
<td>0.51</td>
</tr>
</tbody>
</table>

Data are means± SDs.

<sup>1</sup> Obtained from independent t test.

MUFA, monounsaturated fatty acid; PUFA, polyunsaturated fatty acid; SFA, saturated fatty acid; TDF, total dietary fiber.
Table 3

Means (±standard deviation) of metabolic status at baseline and after the intervention

|                         | Placebo group (n=20) | Probiotic group (n=20) |   |  
|-------------------------|----------------------|------------------------|---|---
|                         | Baseline             | End-of-trial           | Change | P$^1$ | Baseline | End-of-trial | Change | P$^1$ | P$^2$ |
| FPG (mg/dL)             | 89.4±7.8             | 89.3±7.6              | -0.1±7.0 | 0.94 | 102.3±17.7 | 99.7±17.4     | -2.6±9.2 | 0.22 | 0.34 |
| HOMA-B                  | 37.6±15.2            | 47.4±41.6             | 9.8±37.4 | 0.25 | 29.8±19.0  | 22.7±11.9     | -7.1±13.7 | 0.03 | 0.06 |
| QUICKI                  | 0.34±0.02            | 0.34±0.03             | -0.003±0.02 | 0.57 | 0.33±0.02  | 0.34±0.01     | 0.009±0.01 | 0.03 | 0.07 |
| Triglycerides (mg/dL)   | 105.0±42.8           | 111.3±40.0            | 6.3±27.1 | 0.31 | 126.1±69.3 | 134.7±68.3    | 8.6±29.7 | 0.21 | 0.80 |
| VLDL-cholesterol (mg/dL)| 21.0±8.6             | 22.2±8.0              | 1.2±5.4  | 0.31 | 25.2±13.9  | 26.9±13.7     | 1.7±5.9  | 0.21 | 0.80 |
| Total cholesterol (mg/dL)| 184.1±30.3           | 179.6±31.0            | -4.5±20.9 | 0.34 | 174.0±34.4 | 172.5±33.9    | -1.5±21.5 | 0.75 | 0.65 |
| LDL-cholesterol (mg/dL) | 110.9±26.8           | 105.3±27.9            | -5.6±17.4 | 0.16 | 100.9±30.4 | 93.1±30.1     | -7.8±22.4 | 0.13 | 0.74 |
| HDL-cholesterol (mg/dL) | 52.2±12.4            | 52.1±9.4              | -0.1±7.6 | 0.95 | 47.9±11.6  | 52.4±11.3     | 4.5±10.1  | 0.05 | 0.10 |
| TAC (mmol/L)            | 865.9±159.0          | 851.6±155.6           | -14.3±137.2 | 0.64 | 894.9±135.3 | 877.5±87.1    | -17.4±109.9 | 0.48 | 0.93 |

$^1$ Obtained from paired-samples t-tests.

$^2$ Obtained from repeated measures ANOVA test.

FPG, fasting plasma glucose; HOMA-B, homeostatic model assessment-Beta cell function; HDL-cholesterol, high density lipoprotein-cholesterol; LDL-cholesterol, low density lipoprotein-cholesterol; QUICKI, quantitative insulin sensitivity check index; VLDL-cholesterol, very low density lipoprotein-cholesterol; TAC, total antioxidant capacity.
Table 4

Adjusted changes in metabolic variables in patients with MDD that received either probiotic supplements or placebo¹

<table>
<thead>
<tr>
<th></th>
<th>Placebo group (n=20)</th>
<th>Probiotic group (n=20)</th>
<th>P²</th>
</tr>
</thead>
<tbody>
<tr>
<td>BDI total score</td>
<td>-1.8±1.2</td>
<td>-5.3±1.2</td>
<td>0.05</td>
</tr>
<tr>
<td>FPG (mg/dL)</td>
<td>-1.5±1.7</td>
<td>-1.2±1.7</td>
<td>0.92</td>
</tr>
<tr>
<td>Insulin (µU/mL)</td>
<td>2.5±1.6</td>
<td>-2.2±1.6</td>
<td>0.05</td>
</tr>
<tr>
<td>HOMA-IR</td>
<td>5.4±0.4</td>
<td>-0.6±0.4</td>
<td>0.04</td>
</tr>
<tr>
<td>HOMA-B</td>
<td>9.9±6.5</td>
<td>-7.2±6.6</td>
<td>0.08</td>
</tr>
<tr>
<td>QUICKI</td>
<td>-0.001±0.005</td>
<td>0.007±0.005</td>
<td>0.17</td>
</tr>
<tr>
<td>Triglycerides (mg/dL)</td>
<td>5.8±5.9</td>
<td>9.1±5.9</td>
<td>0.69</td>
</tr>
<tr>
<td>VLDL-cholesterol (mg/dL)</td>
<td>1.2±1.2</td>
<td>1.8±1.2</td>
<td>0.69</td>
</tr>
<tr>
<td>Total cholesterol (mg/dL)</td>
<td>-2.8±4.6</td>
<td>-3.2±4.6</td>
<td>0.96</td>
</tr>
<tr>
<td>LDL-cholesterol (mg/dL)</td>
<td>-4.5±4.4</td>
<td>-8.9±4.4</td>
<td>0.49</td>
</tr>
<tr>
<td>HDL-cholesterol (mg/dL)</td>
<td>1.0±1.7</td>
<td>3.4±1.7</td>
<td>0.32</td>
</tr>
<tr>
<td>hs-CRP (ng/mL)</td>
<td>188.5±378.2</td>
<td>-1138.9±378.2</td>
<td>0.01</td>
</tr>
<tr>
<td>TAC (mmol/L)</td>
<td>-21.1±22.9</td>
<td>-10.6±22.9</td>
<td>0.75</td>
</tr>
<tr>
<td>GSH (µmol/L)</td>
<td>-101.6±33.2</td>
<td>-3.4±33.2</td>
<td>0.04</td>
</tr>
</tbody>
</table>

¹ All values are means± SEs. Values are adjusted for baseline values, age and baseline BMI.
² Obtained from ANCOVA test.

BDI, Beck Depression Index; FPG, fasting plasma glucose; GSH, total glutathione; HOMA-IR, homeostasis model of assessment-insulin resistance; HOMA-B, homeostatic model assessment-Beta cell function; HDL-cholesterol, high density lipoprotein-cholesterol; LDL-cholesterol, low density lipoprotein-cholesterol; MDD, major depression disorder; QUICKI, quantitative insulin sensitivity check index; VLDL-cholesterol, very low density lipoprotein-cholesterol; Hs-CRP, high sensitivity C-reactive protein; TAC, total antioxidant capacity.
Fig. 1. Summary of patient flow diagram.
**Fig. 2.** Changes in (means ± standard deviation) of BDI score and metabolic status after 8 weeks of intervention

BDI, Beck Depression Index; HOMA-IR, homeostasis model of assessment-insulin resistance; Hs-CRP, high sensitivity C-reactive protein; GSH, total glutathione.
1. We evaluated effects of probiotic administration on clinical and metabolic responses in patients with major depressive disorder.

2. Probiotic-supplemented patients had beneficial effects on Beck Depression Index (DBI) total score.

3. Probiotic supplementation among patients with MDD had beneficial effects on markers of insulin metabolism.